# Hypolipidaemic Effects of Methanolic Leaf Extract of *Anisopus mannii* in Diet-Induced Hyperlipidemic Albino Rats

.

## **ABSTRACT**

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| **Aim:** to investigate the hypolipidemic effects of the methanolic leaf extract of *Anisopus mannii.*  **Study design:** An *in vivo* experimental study using diet-induced hyperlipidemic albino rats to evaluate the antihyperlipidemic potential of methanolic leaves extract of *Anisopus mannii*.  **Place and Duration of Study:** Department of Biochemistry, Umaru Musa Yar’adua University, Katsina, between September 2023 and March 2024.  **Methodology:** The leaves extract were evaluated for the presence of phytochemicals. The extract was subjected to thin layer chromatography to obtain pure fractions, the fractions obtained were fed to hyperlipidemic albino rats.  **Results:** Phytochemical screening revealed the presence of alkaloids (4.50%), flavonoids (3.69%), saponins (3.26%), phenols (2.50%), terpenoids (1.86%), and tannins (1.56%). The thin layer chromatography revealed four (4) fractions. The lipid-lowering efficacy was evaluated in hyperlipidemic rats over three weeks, with Fraction F2 showing the most promising results. This fraction significantly reduced total cholesterol (46.5 ± 1.6 mg/dl) and triglycerides (72.3 ± 1.1 mg/dl) compared to the hyperlipidemic control (123 ± 1.9 mg/dl and 133.3 ± 3.1 mg/dl respectively), while increasing HDL (46.8 ± 0.1 mg/dl) and reducing LDL (26.2 ± 3.0 mg/dl) levels. These effects were comparable to the standard drug Atorvastatin. GC-MS analysis of the most active fraction revealed the presence of several bioactive compounds, including α-Farnesene, Caryophyllene, and their derivatives, which may contribute to its therapeutic effects.  **Conclusion:** This study demonstrated that the methanolic leaf extract of *Anisopus mannii* possesses lipid lowering effects which could be useful in the treatment of hyperlipidemia. |

*Keywords**: Anisopus mannii*, hyperlipidemia, lipid profile, GC-MS, phytochemicals, albino rats

## **1. Introduction**

For centuries, mankind has depended on the resources provided by the natural world to fulfill their fundamental requirements, particularly in the area of healthcare where natural remedies have been crucial in addressing a diverse range of illnesses [1]. Throughout Africa, plants have served as the foundation of traditional medicine for countless centuries. With a history spanning thousands of years, these plants are recognized as 'Medicinal Plants' due to their possession of therapeutic properties and their ability to produce beneficial pharmacological effects on the bodies of animals [2]. African people hold herbal medicines in high regard, considering them an integral component of their culture and traditions [3]

Musa *et al.* [4] reported that, *Anisopus mannii* which is presently utilized in the traditional medicinal preparations in Northern Nigeria (incomplete sentence). It is a perennial herb that features wide-spreading leaves with a petiole measuring 1.3 - 2 cm in length. At the apex of each leaf, there is a noticeable gland. The blade of the leaf measures approximately 5.7 - 7.6 cm in length. Furthermore, this herb possesses a twining stem that can reach a height ranging from 3.7 to 4.6 meters [2].

The terms 'Sakayau' and 'Kashe zaki,' derived from the Hausa language were assigned to *Anisopus mannii* due to its antidiabetic properties (need reference). These names, which literally mean 'sweet murderer' or 'killing sweetness’, highlight the plant's ability to counteract the effects of excessive sweetness or sugar in the context of managing diabetes [5]. Traditionally, Decoction made from the entire plant, can be employed to treat disease conditions such as parasitic infections, pain relief, inflammation, high blood pressure, microbial infections, and fertility issues [6]. Earlier phytochemical screening of its leaves confirmed the presence of flavonoids, alkaloids, terpenoids, and saponins [7], which are compounds known to influence lipid metabolism. Despite traditional use, scientific validation of its lipid-lowering effect and active chemical constituents remains limited.

Hyperlipidemia is a medical condition characterized by elevated levels of lipids, including cholesterol and triglycerides, in the bloodstream. it is a major risk factor for the development of cardiovascular diseases, including atherosclerosis and coronary artery disease [8].

Hyperlipidemia is broadly categorized into primary and secondary types. Primary hyperlipidemia refers to elevated lipid levels primarily caused by genetic factors, whereas secondary hyperlipidemia is associated with other underlying conditions or lifestyle factors [8].

Hyperlipidemia is a frequently occurring condition; however, a majority of patients remain undiagnosed and, consequently, untreated. The impact of this condition is substantial, resulting in increased morbidity, mortality, and medical expenses. Hyperlipidemia ranks as the second most prevalent risk factor for cardiovascular diseases. When hypertriglyceridemia is combined with high levels of LDL-C, it significantly amplifies the risk of developing coronary heart disease (CHD) [9]. According to WHF [10], raised non-HDL cholesterol was responsible for approximately 4.4 million deaths and 98.6 million disability-adjusted life years (DALYs) globally in 2019.

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## **2. Material and methods**

### **Chemicals and Reagents**

All chemicals and reagents used in this study were of analytical grade.

### **Plant Material**

The fresh leaves of the plant Sample “*Anisopus mannii”* was collected from its habitat at Kurfi local government area of Katsina State. The plant was taxonomically authenticated at Umaru Musa Yar’adua University’s herbarium unit, Biology Department. The plant was given a voucher number (UMYUH8)

### **Preparation of Plant Extract**

The plant leaves were washed with tap water and dried at room temperature, the dried leaves were then grounded to powder using laboratory mortar and pestle, the powdered was then put in a ziplock bag until it was ready for extraction. The Soxhlet extraction method was employed to extract bioactive compounds from dried and ground plant material using methanol as the solvent. Approximately 40g of the plant powder was placed into a thimble made of a soft cloth, which was then inserted into the Soxhlet extractor. The Soxhlet extractor was attached to a round-bottom flask containing 200 mL of methanol and connected to a reflux condenser. The extraction was carried out over a heating mantle set to maintain the boiling point of methanol at around 65°C. The solvent evaporated, condensed, and repeatedly extracted the plant material over a period of 8 hours. After the extraction was complete, the methanolic extract was concentrated using a rotary evaporator at 50°C to remove excess solvent. The concentrated extract was then stored in a glass petri dish in a cool, dark place ready for experiments. This procedure was repeated 4 times to give 200g of powder extracted in 800ml of methanol.

### **Thin Layer Chromatography (TLC) Analysis**

Thin Layer Chromatography (TLC) was carried out following the procedure described by Kidane *et al.* [11], with a few minor adjustments. To begin, 1 mg/mL of the plant extract (dissolved in methanol) was carefully applied onto a silica gel-coated TLC plate, about 1 cm from the bottom edge, using a micropipette. After allowing the spot to dry, the plate was gently placed in a solvent mixture made of equal parts ethyl acetate and methanol (5 mL each). Once the solvent front rose to about three-quarters of the plate, it was taken out and dried briefly at a moderate temperature range of 60–120°C. The separated bands on the plate were then individually scraped into labelled beakers and washed with methanol to isolate the fractions. These fractions were dried in a hot air oven and stored until they were needed for further testing. The entire process was repeated three times to ensure reliability. Retention factor (Rf) values of the fractions were calculated using:

Rf = Distance travelled by the sample/ Distance travelled by the solvent

### **Induction of hyperlipidemia**

Atherogenic diet was formulated using 70% super starter feed, 25% Palm oil and 5% egg York. Constantly feeding the rats for 7 weeks induced hyperlipidemia [12].

### **Experimental design**

The Albino rats were grouped into seven groups of five rats each, fed for 7 weeks and the treatment was administered for 3 weeks. The weight of the rats was constantly taken for each week.

Grouping

* Group A: Normal control group (no treatment) given Animal feed and water
* Group B: Hyperlipidemic control group (no treatment) given Atherogenic diet and water
* Group C: Standard drug control group (Statin drug treatment) given Atherogenic diet and water
* Group D: Extract control group (50mg/kg of fraction 1) given Atherogenic diet and water
* Group E: Extract control group (50mg/kg of fraction 2) given Atherogenic diet and water
* Group F: Extract control group (50mg/kg of fraction 3) given Atherogenic diet and water
* Group G: Extract control group (50mg/kg of fraction 4) given Atherogenic diet and water

### **Determination of the blood lipid profiles**

Include statement here regarding handling of experimental animals per international conventions https://www.elgaronline.com/edcollchap/book/9781803923673/chapter13.xml#:~:text=Directive%202010/63%20restricts%20the,are%20exempted%20under%20the%20Directive. Blood samples were collected after an overnight fast of 10-12 hours. Chloroform served as a mild anesthetic for the rats, and blood was drawn via cardiac puncture using a 5 ml syringe. To prevent clotting, the blood samples were placed in Lithium-heparin tubes. These samples were then centrifuged at 3000 rpm, and the resulting serum was collected in clean containers for lipid profile tests. The levels of Serum total glyceride (TG), High-density lipoprotein Cholesterol (HDL), and Total Cholesterol (TC) were estimated using the instructions provided with the commercial kits. Low-density lipoprotein cholesterol (LDL) and were estimated using the Friedewald formula [13].

### **Characterization of the most active fraction of Anisopus mannii using gas chromatography- mass spectroscopy (GCMS)**

Gas Chromatography-Mass Spectrometry (GC-MS) was utilized to determine the chemical composition of the sample through a series of systematic steps.

Sample Preparation involved dissolving the dried extract in an appropriate solvent, typically methanol or dichloromethane, to create a solution. This solution was filtered using a 0.45 µm membrane filter to remove particulate matter prior to injection into the GC-MS system [14]

Instrumentation included the use of an Agilent Technologies 7890A GC system integrated with a 5975C VL Mass Selective Detector (MSD) equipped with a Triple-Axis Detector. The system utilized an HP-5 MS capillary column measuring 30 m in length, 0.25 mm in internal diameter, and with a film thickness of 0.25 µm.

**GC Conditions** were meticulously configured, starting with an oven temperature of 60°C held for 2 minutes. The temperature was then increased at a rate of 10°C per minute until reaching 280°C, where it was held for 10 minutes, making the total run time 32 minutes. The injector temperature was maintained at 250°C, with helium as the carrier gas at a constant flow rate of 1.0 mL/min. A 1 µL injection volume was applied in splitless mode [15]

MS Conditions included operating the mass spectrometer in electron ionization (EI) mode at 70 eV. The ion source temperature was set to 230°C, and the quadrupole temperature was maintained at 150°C. The system scanned a mass range of 50 to 550 m/z in full scan mode to identify the sample's chemical constituents, with data acquisition supported by the NIST library for compound identification [16]

### **Statistical Analysis**

Statistical analysis was performed using Graph Pad Instat3 Software version, 3.05. One-way analysis of variance (ANOVA) was used to evaluate the statistical differences between different inhibitory concentrations (?means) followed by Tukey HSD post hoc test. values were considered significant when P<0.05. Experiments were done in triplicate and the mean value was reported as mean ± S.D.

## **3. Results and discussion**

### **Qualitative Phytochemical Analysis**

Qualitative phytochemical tests for the methanolic leaves extract of *Anisopus mannii* revealed the presence of flavonoids, alkaloids, tannins, phenols, saponins and terpenoids. However, glycosides, cardiac glycosides, quinones and steroids were not detected (Table 1).

Table 1: Qualitative Phytochemical Content of the methanolic Leaf Extract of *Anisopus mannii*

|  |  |
| --- | --- |
| Phytochemicals Tested | Inference |
| Flavonoids | + |
| Tannins | + |
| Phenols | + |
| Alkaloids | + |
| Saponins | + |
| Steroids | - |
| Terpenoids | + |
| Cardiac glycosides  Quinones  Glycosides | -  -  - |

Key: (+) detected, (-) not detected

### **Quantitative Phytochemical Analysis**

Table 2 presents the quantitative analysis of phytochemical constituents present in *A. mannii* leaf extract showed that Alkaloid has the highest value of 4.5%, Flavonoids was calculated to be 3.69%, Total phenols have 2.50%, Total Tannins has lowest value of 1.56%, total Terpenoids has 1.86% and total Saponins was calculated to be 3.26%.

Table 2: Quantitative Phytochemical Composition of the Methanolic Leaf Extract of *Anisopus mannii*

|  |  |
| --- | --- |
| **Test** | ***A. mannii* leave (%)** |
| Alkaloids | 4.50 |
| Flavonoids | 3.69 |
| Phenols | 2.50 |
| Tannins | 1.56 |
| Saponins | 3.26 |
| Terpenoids | 1.86 |

Key; % Percentage

### **Thin Layer Chromatography**

The thin layer chromatography (TLC) OF *A. mannii* was conducted and yielded four (4) separate fractions. The fractions revealed four different shades of colors, from dark brown, light brown, light green and dark green, this is an indication of the presence of different phytochemical compounds. The differences in color indicate variations in the chemical composition or the concentrations of specific components. The Retention factor for the four fractions was calculated to be equally 0.53±0.01 respectively. This may suggest that the components in the extract likely possess comparable polarities or have similar interactions with the stationary phase.

Table 3: Fractions of the Methanolic Leaf extract of *Anisopus mannii*

|  |  |
| --- | --- |
| **Fractions** | **Retention factor (Rf)** |
| Fraction 1 | 0.53±0.01 |
| Fraction 2 | 0.53±0.01 |
| Fraction 3 | 0.53±0.01 |
| Fraction 4 | 0.53±0.01 |

Data are presented as mean ± standard deviation of triplicate readings (n=3).

### **Lipid-lowering effect of the methanolic leaf extract of *Anisopus mannii* in hyperlipidemic albino rats.**

Administration of 50 mg/kg of methanolic leaf extract fractions of *Anisopus mannii* significantly improved lipid profiles in high-fat diet-fed rats (p < 0.05). Fraction F2 showed the strongest effect, reducing total cholesterol, triglycerides, and LDL From?? to 46.5 ± 1.6, 72.3 ± 1.1, and 26.2 ± 3.0 mg/dl, respectively, while increasing HDL to 46.8 ± 0.1 mg/dl from ??

Other fractions (F1, F3, F4) also improved lipid parameters, though to a lesser extent. The performance of Fraction F2 was comparable to Atorvastatin, underscoring its potential as a natural antihyperlipidemic agent.

**Table ‎4:** Lipid-lowering effect of the methanolic leave extract of Anisopus mannii in hyperlipidemic Albino Rats.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Group** | **TC (mg/dl)** | **TG (mg/dl)** | **HDL (mg/dl)** | **LDL (mg/dl)** |
| N.C | 75.0 ± 0.7a | 84.3 ± 0.9a | 49.0 ± 1.6a | 49.0 ± 0.7a |
| H.C | 123.0 ± 1.9b | 133.3 ± 3.1b | 23.7 ± 0.4b | 59.7 ± 1.3b |
| SD.C | 45.7 ± 0.3bc | 51.3 ± 1.2c | 35.3 ± 0.7c | 26.0 ± 1.4c |
| F1(50mg/kg) | 47.1 ± 2.1c | 74.5 ± 1.5a | 42.0 ± 3.0a | 28.9 ± 2.1c |
| F2 (50mg/kg)  F3 (50mg/kg)  F4 (50mg/kg) | 46.5 ± 1.6c  66.0 ± 0.8a  50.0 ±1.1c | 72.3 ± 1.1a  84.7 ± 2.6a  79.7 ± 2.4a | 46.8 ± 0.1a  39.0 ± 0.4c  49.7 ± 1.6a | 26.2 ± 3.0c  29.0 ± 1.6c  26.7 ± 0.8c |

Values are expressed as mean ± standard deviation. Different lowercase superscripts in the same column are significantly different at *P <0.05.*

Key: N.C = Normal control, H.C= Hyperlipidemic control, SD.C= Standard drug control, F = Fractions, TC= Total cholesterol, TG= Triglycerides, HDL= High density lipoprotein, LDL= Low density lipoprotein.

### **Characterization of the most active fraction of *Anisopus mannii* using gas chromatography mass spectroscopy (GCMS)**

Table 5 shows the GC-MS analysis of the most active fraction, Fraction 2. it identified six major compounds with high confidence, including sesquiterpenes like (Z,Z)-α-Farnesene, Caryophyllene, Eremophilene, and Caryophyllene oxide, known for anti-inflammatory and antimicrobial properties. Also detected were 2,5-Pyrrolidinedione and a pyridinium derivative, suggesting additional pharmacological potential of the fraction.

**Table 5**: GC-MS Analysis of A. mannii representing fraction 2

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Peak Number** | |  | | --- | | **Compound Name** |  |  | | --- | |  | | **Chemical formular** | |  | | --- | | **Molecular Weight (MW)** |  |  | | --- | |  | | **Confidence level** |
| 2 | |  | | --- | | (Z,Z)-α-Farnesene | | |  | | --- | | C15H24 | | |  | | --- | | 204 | | High |
| 3 | |  | | --- | | Caryophyllene | | |  | | --- | | C15H24 | | |  | | --- | | 204 | | High |
| 7 | Eremophilene | |  | | --- | | C15H24 |  |  | | --- | |  | | 204 | High |
| 13 | |  | | --- | | Caryophyllene oxide | | |  | | --- | | C15H24O | | 220 | High |
| 18 | 2,5-Pyrrolidinedione, 1-[(3,4-dimethylbenzoyl)oxy]- | C13H13NO4 | 247 | high |
| 23 | Pyridinium, 1-ethyl-, hydroxide | C7H11NO | 125 | High |

### **The key summary of Fraction 2**

This sample contains mostly terpenoids and aromatic compounds.

1. **(Z,Z)-α-Farnesene**
   * **Class**: Sesquiterpene
   * **Description**: A naturally occurring terpene with a fruity aroma.
   * **Usage**: Found in the coatings of apples and other plants, also used in perfumes and flavoring.
2. **Caryophyllene**
   * **Class**: Sesquiterpene
   * **Description**: A bicyclic sesquiterpene with spicy, woody aroma.
   * **Usage**: Used in essential oils, flavoring, and as an anti-inflammatory agent.
3. **Eremophilene**
   * **Class**: Sesquiterpene
   * **Description**: Another member of the sesquiterpene family, often derived from plants.
   * **Usage**: Found in essential oils, with potential applications in aromatherapy and fragrances.
4. **2,5-Pyrrolidinedione, 1-[(3,4-dimethylbenzoyl)oxy]-**
   * **Class**: Pyrrolidinedione derivative
   * **Description**: A nitrogen-containing cyclic compound with potential pharmaceutical uses.
   * **Usage**: It may serve as an intermediate in drug synthesis or specialty chemicals.
5. **Pyridinium, 1-ethyl-, hydroxide**
   * **Class**: Pyridinium salt
   * **Description**: A positively charged nitrogen-containing heterocyclic compound.
   * **Usage**: It can be used as a phase-transfer catalyst or in synthetic organic chemistry.

## **4. DISCUSSION**

1st paragraph of the discussion should be about your results. Discuss your results,not reviewing the literature. Use the literature to support your results. You have important results but I do not see you discussing the results here in the discussion.

Phytochemical analysis plays a crucial role in phytomedicine by identifying bioactive compounds responsible for therapeutic effects [17]. The qualitative phytochemical screening in this study revealed the presence of several bioactive compounds including alkaloids, flavonoids, saponins, tannins, terpenoids, and phenols, this is supported by the work of name the author?? [7] where they reported that the crude methanol extract/solvent fractions of *A. mannii* were found to contain phytochemicals including flavonoids, saponins, tannins, triterpenes, cardiac glycosides, and alkaloid. Also, standard phytochemical screening techniques have identified a range of compounds, including alkaloids, flavonoids, tannins, saponins, glycosides, phenols, and terpenes, among others in *A. mannii* [18]. But, the absence of glycosides, cardiac glycosides, steroids, and quinones indicates a selective phytochemical profile which distinguishes the methanolic leaf extract with ethanolic stem extract. Also, Osuntokun *et al.* [2]confirmed the presence of Cardiac glycoside in both the leaves and bark of *Anisopus mannii* ethyl acetate extracts which is in contrast to this study.

The Thin Layer Chromatography (TLC) analysis of *Anisopus mannii* leaf extract yielded four distinct fractions with similar retention factors (0.53 ± 0.01), indicating the presence of compounds with comparable polarities.

Subsequent Gas Chromatography-Mass Spectrometry (GC-MS) analysis of these fractions revealed a complex mixture of bioactive compounds, notably rich in terpenoids and phenolic compounds. Among these, compounds such as α-Farnesene and Caryophyllene were identified in Fraction 2. These terpenoids are known for their therapeutic properties, including anti-inflammatory, antimicrobial, and sedative effects. For instance, α-Farnesene has been associated with anti-inflammatory and antimicrobial activities [19] while β-Caryophyllene is recognized for its antinociceptive and anti-inflammatory effects [20]. The identification of these compounds within *A. mannii* leaf extract underscores the plant's potential as a source of natural therapeutic agents. The presence of terpenoids and phenolic compounds contributes to the extract's bioactivity, supporting its traditional use in herbal medicine.

2nd 3rd, and 4th paragraphs provide some explaination to the results of your study? Propose any biochemical mechanisms. Cite other literature to support or propose biochemical pathways or mechanisms to explain your results.

The superior performance of Fraction F2 is strongly associated with its terpenoid content, including compounds like Caryophyllene oxide and α-Farnesene, identified through GC-MS analysis. Caryophyllene oxide has been previously documented for its anti-inflammatory and metabolic regulatory effects [21]. These mechanisms, involving enhanced lipid metabolism and reduced inflammation, could explain the observed hypolipidemic effects. Such findings are in line with other research on plant-based lipid-lowering agents. For example, a study by Kamran *et al*. [22] demonstrated the efficacy of bioactive terpenoids in regulating lipid profiles and reducing oxidative stress, supporting their potential as therapeutic agents. Similarly, phenolic compounds in plant extracts have been associated with lipid-lowering and anti-inflammatory activities [23].

One paragraph should be dedicated to highlighting the limitations of your study. And propose areas for further research based on what you found.

## **Conclusion**

Based on the findings in this study, it can be concluded that *A. mannii* leaf extract possesses significant potential as a natural lipid-lowering agent. its therapeutic efficacy, makes it a promising candidate for further development in the management of hyperlipidemia. However, further research is recommended to elucidate the exact mechanisms of action and to develop standardized formulations for therapeutic use.

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